California Sea Grant College Program
Progress Report

Project Information

Year

Number R/NMP-100

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Start Date: 2/1/2010 **Completion Date:** 7/31/2013

Title Exploiting marine actinomycete diversity for natural product discovery

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Project Hypothesis

The project hypotheses have not changed. We continue to address questions related to the diversity and distributions of MAR4 actinomycetes in marine sediments, the relationships between who these bacteria are (phylogeny), the types of secondary metabolites they produce, and the mechanistic biochemistry responsible for their biosynthesis.

Project Goals and Objectives

The ultimate objective of this project is to discover new and structurally diverse hybrid isoprenoid (HI) antibiotics from marine actinomycetes. The research focuses on an unusual group of marine actinomycetes that were discovered in the PIs laboratory and have tentatively been called MAR4. For reasons that are not yet entirely clear, MAR4 strains have proven to be a rich source of HI secondary metabolites, which are generally rare among bacteria. This proposal takes a unique approach that couples creative culturing techniques and poorly studied environmental resources with molecular approaches to rapidly identify strains capable of producing HI secondary metabolites. These strains then become the focus of detailed structure elucidation and biosynthetic studies that will reveal the mechanisms by which the compounds are assembled. The enzymes discovered as part of these studies subsequently have the potential to be used as biochemical tools to create additional structural diversity. The proposed studies target the discovery of a wide range of HI secondary metabolites including compounds with N and C-prenylation, prenylated pyroles, phenazines, and peptides. The results may include the discovery of diverse new chemical structures within the broad HI class. Major objectives include detailed phylogenetic analyses of MAR4 strains, which will expand our understanding of the diversity of bacteria that produce HIs. A related objective is the biochemical characterization of gene clusters associated with HI biosynthesis and the cloning of specific

enzymes for use as biochemical tools.

The project goals remained the same during this reporting period. These include an assessment of the diversity of MAR4 marine actinomyetes in ocean sediments collected off the coast of California using both culture and culture independent methods. Goals include the collection of new sediment samples and attempts to draw correlations between the abundance of MAR4 strains and environmental parameters. Additional goals include the identification of hybrid isoprenoid (HI) secondary metabolites produced by MAR4 strains and analyses of the relationships between MAR4 phylotype and the suite of secondary metabolites produced. We continue to work on pathways associated with HI biosynthesis, with a focus on strain CNH-189, which produces the unusual merochlorin class of compounds.

Briefly describe project methodology

The research proposed here applies state of the art methods in microbiology, natural products chemistry, and the molecular genetics of natural product biosynthesis to discover new antibiotics from poorly studied marine microbial resources. In brief, samples collected off the coast of California are being processed using cultivation methods that are selective for the MAR4 group of marine actinomycetes. The taxonomic diversity of these strains is being examined by 16S gene sequencing while their ability to produce HI secondary metabolites is analyzed by LC-MS following cultivation and extraction. In addition, culture-independent methods are being used to assess total MAR4 diversity, which can then be used to gauge the effectiveness of the culture-based techniques.

During this reporting period, we participated in two 7-day cruises aboard the R/V Melville off the coast of San Diego. These cruises provided access to large numbers of deep-sea sediment cores. Each core was sectioned and subsamples preserved for future cultivation and DNA work. Considerable environmental data was also collected at each site, which will provide the first opportunity to link MAR4 abundance and distributions to environmental parameters.

We have focused our biosynthetic efforts on the pathway associated with the production of compounds in the merochlorin class during this reporting period. We have established a heterologous expression system that now allows a detailed investigation of this pathway. We have replaced every gene that appears to be involved in merochlorin biosynthesis with an apramaycin resistance cassette using Red/ET-recombineering. The resistance cassettes have then been eliminated with the help of the Flp-recombinase to produce non-polar gene deletions. The mutant fosmids were transferred into S. coelicolor M1152 for heterologous expression. This comprehensive set of knock-outs will be analyzed by a combination of UV-assisted HPLC, LC-HR-MS/MS and NMR spectroscopy.

Describe progress and accomplishments toward meeting goals and objectives.

A unique opportunity to participate in two, one-week research cruises aboard the R/V Melville off the coast of California yielded a large number of deep-sea sediment cores that are being processed for MAR4 diversity studies. Along with these samples, considerable environmental data was collected, which will provide the first opportunity to correlate the distribution of these bacteria with environmental parameters. We are nearing completion of a manuscript describing the cultured and culture-independent (CI) diversity of MAR strain strains in marine sediments collected off the Channel Islands, CA. These studies revealed that considerable MAR4 diversity remains uncultured and that some of the cultured strains were not observed in the CI analyses. This later results demonstrates the importance of applying both techniques. The phylogeny of the MAR4 group reveals two primary clades, each of which is characterized by the types of HI secondary metabolites they produce. Once the biochemical analyses are completed, a manuscript describing the merochlorin biosynthetic pathway will be prepared.

PROJECT MODIFICATIONS: Explain briefly any substantial modifications in research plans, including new directions pursued and ancillary research topics developed. Describe major problems encountered and how they were resolved.

No modifications to report.

PROJECT OUTCOMES: Briefly describe data, databases, physical collections, intellectual property, models, instruments, equipment, techniques, etc., developed as a result of this project and how they are being shared.

IMPACTS OF PROJECT: Briefly describe how this project has contributed to a discipline; to developing human resources; to developing physical, institutional or information resources; technology transfer; and society beyond science and technology. Please notify CASG of impacts that occur after your project ends; CASG may contact you after your project ends to learn about additional impacts that occur over time.

BENEFITS, COMMERCIALIZATION, AND APPLICATION OF PROJECT RESULTS: Please list any companies, agencies, organizations or individuals who have used your project results, scientific/technical advice, etc., and provide names, emails and phone numbers. Briefly describe how results were used and quantify results and socioeconomic benefits, if possible.

ECONOMIC BENEFITS generated by discovery, exploration and development of new, sustainable coastal, ocean and aquatic resources (i.e., aquaculture, marine natural products, foods, pharmaceuticals).

Issue-based **forecast capabilities** to predict the impacts of a single ecosystem stressor, developed and used for management (i.e., climate change,

extreme natural events, pollution, invasive species, and land resource use).

Tools, technologies and **information services** developed (i.e., land cover data, benthic habitat maps, environmental sensitivity index maps, remove sensing, biosensors, AUVs, genetic markers, technical assistance, educational materials, curricula, training).

Publications (list in appropriate category below) Each listing should be a stand-alone bibliographic reference, including all authors' names. For each Publication type, specify title, authors, date and journal details, where appropriate (repeat headers as necessary).

Technical Reports

Title	Authors	Date

Conference Papers, Proceedings, Symposia

Peer-reviewed journal articles or book chapters

Journal J. Amer. Chem. Soc. **Issue Num** 134 **Page Num** 11988 **Date** 2012

Title Merochlorins A-D, cyclic meroterpenoid antibiotics biosynthesized in divergent pathways with vanadium-dependent chloroperoxidases.

Authors Kaysser L, Bernhardt P, Nam S-J, Loesgen S, Ruby, J, Skewes-Cox P, Jensen PR, Fenical W, Moore BS.

Journal Antonie van Leeuwenhoek **Issue Num** **Page Num** early on **Date** 2012

Title Marine actinobacteria from the Gulf of California: Diversity, abundance and secondary metabolite biosynthetic potential.

Authors Becerril-Espinosa A, Freel KC, Jensen PR, Soria-Mercado IE

Journal FEMS Microbial Ecology **Issue Num** **Page Num** accepted **Date** 2013

Title Targeted search for actinomycetes from near-shore and deep-sea marine sediments.

Authors Prieto-Davo A, Villarreal-G_omez LJ, Forschner-Dancause S, Bull AT, Stach JEM, DC, Rowley DC, Jensen PR

Non-peer Reviewed Reprints

Publications, Brochures, Fact Sheets

Books & Monographs

Handbooks, Manuals, Guides

Electronic publications: (non-print formats).

Maps, Charts, Atlases

Theses, dissertations

Newsletters, periodicals

Program reports (annual/biennial, strategic plans, implementation plans)

Educational Documents

Topical Websites and Blogs

Miscellaneous documents (not listed above).

MEDIA COVERAGE: Select 'Yes' or 'No'. If yes, describe any radio, TV, web site, newspaper, magazine coverage your project has received. Send original clippings or photocopies to the Sea Grant Communications Office.

MEDIA NOTES: Brief description of the type media coverage your project has received.

DISSEMINATION OF RESULTS: List any other ways in which results of your project have been disseminated. Indicate targeted audiences, location, date and method.

WORKSHOPS AND PRESENTATIONS: A brief description of location, date, time, topic, number of attendees and name of presenter.

COOPERATING ORGANIZATIONS: List those (e.g., county or state agencies, etc.) who provided financial, technical or other assistance to your project since its inception. Describe the nature of their cooperation.

Federal Organizations

Regional Organizations

State Organizations

Nongovernment Organizations

International Organizations

Industry Organizations

Academic Organizations

Sea Grant Organizations

Other Organizations

INTERNATIONAL IMPLICATIONS: Does your project involve any colleagues overseas or have international implications?

AWARDS: List any special awards or honors that you, or any co-project leaders, have received during the duration of this project.

KEYWORDS: List keywords that will be useful in indexing your project.

natural product discovery

PATENTS: Please list any patents or patent licenses that have resulted from this project, and complete the patent statement form available on the web site.

NOTES: Please list any additional information in the notes area

FOR ALL STUDENTS SUPPORTED BY THIS GRANT, PLEASE LIST:

Volunteer Count

Graduate Student Info